REMARKS

Reconsideration of this application is requested. Claims 24-30, 36, 37 and 40 are in the case.

I. SPECIFICATION

The Abstract has been objected to as including inappropriate language. In response, a new Abstract is presented on a separate sheet attached to this response.

C3 attach.

II. THE 35 U.S.C. § 112, FIRST PARAGRAPH, REJECTION

Claims 24-30, 36, 37 and 40 stand rejected under 35 U.S.C. § 112, first paragraph, on the grounds that the specification, while enabling for ubiquitination of a protein using a compound in a cell lysate assay system, allegedly does not reasonably provide enablement for ubiquitination of a compound within a cell, or for the use of the compound as a pharmacological agent in a patient. The specification is alleged to be non-enabling with respect to practice of the invention commensurate in scope with the claims in the case.

Claim 24, as now amended, is directed to a method for reducing the level and/or activity of a target protein in a eukaryotic cell via the activation of ubiquitination of the target protein. The method comprises contacting the cell with a compound comprising:

c) a ubiquitination recognition element which is able to bind to either the E3 or E2 elements of the ubiquitination system, wherein the ubiquitination recognition element has a molecular weight less than 30,000 and has a

binding affinity for the E3 and/or E2 elements of the ubiquitination system of at least 10^3 M^{-1} and;

d) a target protein binding element that is able to bind specifically to the target protein wherein the target protein binding element has a molecular weight of less than 30,000 and has a binding affinity for the target protein greater than 10^5 M^{-1}.

The ubiquitination recognition element is covalently linked to the target protein binding element.

The amendment to claim 24 changes 10^2 M^{-1} to 10^3 M^{-1} in respect of the binding affinity of the ubiquitination recognition element. New claim 43 defines the molecular weight of the ubiquitination recognition element as between 50 and 10,000. Basis for these amendments appears in original claim 5.

The claimed invention is fully enabled by the specification, and a person of ordinary skill in this art would not consider recovering activity within a cell to be "undue experimentation". The reasons for this are as follows.

The Examiner has identified the factors to be considered in determining whether undue experimentation is required, as summarized in *In re Wands*, 858 F.2d 731,8 USPQ2d 1400 (CAFC 1988). The Examiner's comments appear to focus on three areas, namely affinity of the compounds, cell take-up and degradation. The following comments are offered.

1. Affinity of the Compounds

The Examiner expresses concern over the issue of non-specific binding from two points of view, namely alternative binding sites and lack of strength to the specific sites. The phenomenon of alternative binding sites is observed with many drugs, but does not prevent the development of effective drugs. Moreover, non-specific binding is a common observation with many drugs that, nevertheless, prove to have valuable in-cell biological activity. For example, data from Sertraline produced by UNICHEM Laboratories Limited "Protein Binding: In vitro protein binding studies performed with radiolabeled 3H-sertraline, showed that sertraline is highly bound to serum proteins (98%) in the range of 20 to 500 ng/mL. However, at up to 300 and 200 ng/mL concentrations, respectively, sertraline and N-desmethylsertraline did not alter the plasma protein binding of two other highly protein bound drugs, viz., warfarin and propranolol". The inventor of the present application has observed that issues of non-specific binding are generally managed with routine dose changes.

With regard to binding affinity, while it is believed that the claims prior to the present amendment are enabled by the present specification, in order to advance prosecution and reduce the issues, claim 24 as amended now recites the binding affinity of the ubiquitination element as 10^3 M^{-1}, rather than 10^2 M^{-1}. It is believed this 10-fold increase in the recited binding affinity addresses the Examiner's concern expressed towards the end of the first complete paragraph on page 5 (and elsewhere) of the action. A ubiquitination recognition element having a binding affinity of 10^3 M^{-1} is capable of interacting with E2 or E3 with high specificity.

2. Cell Up-Take

Many compounds that prove to be valuable pharmacological agents have poor or very low cellular up-take, yet are able to function as valuable pharmaceutical agents. In addition, many methods are known to persons skilled in the art for the introduction of compounds into cells, and do not represent undue experimentation. These methods include chemical methods based on liposomes and membrane transporting peptides. These reagents are widely available (In vitrogen, Active Motif, Santa Cruz), and have been extensively used in the literature. Methods based on the use of viral vectors have also been employed.

An alternative method for introducing molecules into cells is to make use of electroporation or other physical methods. These physical methods are made more facile by the use of *ex vivo* methods, and are well known in the art.

The specification provides specific teachings with respect to these methods, as are known to those skilled in the art. No lack of enablement arises in this regard.

Examples based on teachings from the specification include the following references:

Biochim Biophys Acta 2001 Dec 1;1515(2):101-9: Cargo delivery kinetics of cell-penetrating peptides. Hallbrink M, Floren A, Elmquist A, Pooga M, Bartfai T, Langel U. Department of Neurochemistry and Neurotoxicology, Arrhenius Laboratories, Stockholm University, Sweden.

In summary, this paper reports a diversity of cell-penetrating peptides (CPPs), but so far the only common denominator for these peptides is the ability to gain cell entry in an energy-independent manner. As the mechanism used by CPPs for cell entry is largely unknown, and data comparing the different peptides are lacking, the cellular uptake and cargo delivery kinetics of penetratin, transportan, Tat (48-60) and MAP (KLAL) were studied. The respective CPPs (labelled with the fluorescence quencher, 3-nitrotyrosine) are coupled to a small pentapeptide cargo (labelled with the 2-amino benzoic acid fluorophore) via a disulfide bond. The cellular uptake of the cargo is registered as an increase in fluorescence intensity when the disulfide bond of the CPP-S-S-cargo construct is reduced in the intracellular milieu. The data show that MAP has the fastest uptake, followed by transportan, Tat(48-60) and, last, penetratin.

FASEB J 1998 Jan;12(1):67-77: Cell penetration by transportan. Pooga M, Hallbrink M, Zorko M, Langel U. Department of Neurochemistry and Neurotoxicology, Stockholm University, Sweden.

In summary, this paper reports transportan (a 27 amino acid-long peptide containing 12 functional amino acids from the amino terminus of the neuropeptide galanin and mastoparan in the carboxyl terminus, connected via a lysine) is a cell-penetrating peptide as determined by indirect immunofluorescence using N epsilon13-biotinyl-transportan. The internalization of biotinyl-transportan is energy independent and takes place efficiently at 37º, 4º, and 0º C. Cellular uptake of transportan is probably not mediated by endocytosis, since it cannot be blocked by treating the cells with phenylarsine oxide or hyperosmolar sucrose solution and is nonsaturable. The cell-penetrating ability of transportan is not restricted by cell type, but seems to be a

general feature of this peptide. In Bowes' melanoma cells, transportan first localizes in the outer membrane and cytoplasmatic membrane structures. This is followed by a redistribution into the nuclear membrane and uptake into the nuclei where transportan concentrates in distinct substructures, probably the nucleoli.

Neuroreport 2001 Mar 5;12(3):607-10 Vesicle-associated proteins and transmitter release from sympathetic ganglionic boutons. Blair DH, Robson S, King G, Bennett MR. Neurobiology Laboratory, University of Sydney, NSW, Australia.

A method is reported for introducing peptides derived from SNARE proteins that control exocytosis of vesicles at boutons formed by sympathetic ganglion cells in tissue culture. These peptides were coupled to the DNA binding domain of the Drosophila transcription factor antennapedia, called penetratin. This facilitated the passage of peptides across the bouton membrane. FM1-43 was used to monitor the exocytosis of transmitter from depolarized boutons after their exposure to the penetratin-peptide sequences. This report introduces a readily applicable method for determining the effect of different peptide sequences of vesicle-associated proteins on secretion at vertebrate boutons and presents an account of the effects of a selection of such peptides on exocytosis.

In light of the above, it is believed clear that, in regard to cell up-take, the person of ordinary skill in this art would not have to resort to undue experimentation in carrying out the method as claimed in the present application.

Issues relating to degradation are important but do not negate the potential value or utility of the compounds employed in the method of the invention. It is known to persons of ordinary skill in this art that the vast majority of viable drugs on the market are subject to numerous routes of degradation and elimination, which work to lower the effective levels of the drug. In fact, if a drug were not degraded and eliminated, it would not be an ideal candidate, as it would not be possible to reverse the actions of that drug. Typically, an ideal drug would have a ½ life in cell or in body of 12-24 hours, to provide for a simple dosing means based on one or two doses per day to maintain a level known to be effective in treatment. Degradation or elimination provides an important route to terminate the treatment. Example of drugs that have a very short life and are degraded very rapidly include inhaled glucocorticosteroids such as Budesonide which has a half life of 2-3 hours, and availability of only 6-13% (Barnes PJ, et al. Am J Respir Crit Care Med 1998; 157: S1–53).

In view of the above it is believed clear that the issue of degradation of the compounds is well known to persons of ordinary skill and that one of ordinary skill would not have to undertake undue experimentation with respect to that phenomenon.

In summary, application of the *Wands* factors (the nature of the invention, the state of the prior art, the presence or absence of working examples, the amount or direction or guidance presented, the quantity of experimentation necessary, the relative skill of those in the art, the predictability or unpredictability of the art, and the breadth of the claims) to the present invention as now claimed would not create a situation where

the reader is required to undertake undue experimentation to carry out the claimed invention. While some experimentation may be required, such experimentation is acceptable under the law provided it does rise to the level of being undue.

The method as now claimed in this application is fully enabled by the specification. Reconsideration and withdrawal of the outstanding 35 U.S.C. § 112, first paragraph, rejection are respectfully requested.

Allowance of the application is awaited.

Attached hereto is a marked-up version of the changes made to the abstract by the current amendment. The attached pages are captioned "**Version With Markings To Show Changes Made.**"

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____
Leonard C. Mitchard
Reg. No. 29,009

LCM:lfm
1100 North Glebe Road, 8th Floor
Arlington, VA 22201-4714
Telephone: (703) 816-4000
Facsimile: (703) 816-4100

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS

24. (Twice amended) A method of reducing the level and/or activity of a target protein in an eukaryotic cell via the activation of ubiquitination of said target protein comprising contacting said cell with a compound comprising;

e) a ubiquitination recognition element which is able to bind to either the E3 or E2 elements of the ubiquitination system, wherein said ubiquitination recognition element has a molecular weight less than 30,000 and has a binding affinity for said E3 and/or E2 elements of the ubiquitination system of at least [10^2 M^{-1}] 10^3 M^{-1} and;

f) a target protein binding element that is able to bind specifically to said target protein wherein said target protein binding element has a molecular weight of less than 30,000 and has a binding affinity for said target protein greater than 10^5 M^{-1},

wherein said ubiquitination recognition element is covalently linked to said target protein binding element.

ABSTRACT OF THE DISCLOSURE

Method for reducing the level and/or activity of a target protein in a eukaryotic cell via activation of ubiquitination of the target protein wherein the cell is contacted with the compound having a ubiquitination recognition element covalently linked to a target protein binding element. The ubiquitination and recognition element can bind to either the E3 or E2 elements of the ubiquitination system and the target protein binding element is able to bind specifically to the target protein. The target protein binding element has a molecular weight of less than 30,000 and has a binding affinity for the target protein greater than $10^5 M^{-1}$.